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(57) Abstract

Polynucleotides encoding TAR-1 and TAR-3 receptor polypeptides, as well as such polypeptides, antibodies and antagonists against such polypeptides are disclosed. Also disclosed is a procedure forproducing such polypeptides by recombinant techniques. Use of agonists and antagonists for therapeutic purposes, for example, to inhibit growth of cells and to treat lung and liver fibrosis is also disclosed.

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TGF-β1, ACTIVIN RECEPTORS 1 AND 3

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is TGF-β1, Activin Receptors 1 and 3, sometimes hereinafter referred to as "TAR-1 and TAR-3". The invention also relates to inhibiting the action of such polypeptides.

The cloning of TGF-β1 and the resultant elucidation of its precursor structure (Derynck, et al., Nature, 316:701-705 (1985)) have led to the identification of at least four other forms of TGF-β and the definition of a larger gene family comprising many other structurally related, but functionally distinct, regulatory proteins.

There are now many polypeptides that belong to the TGF-β supergene family by virtue of amino acid homologies, particularly with respect to the conservation of seven of the nine cysteine residues of TGF-β among all known family members. These include the mammalian inhibins (Mason, et al., Nature, 318:659-663 (1985)) and activins (Ling, et al., Nature, 779-782 (1986)), and mullerian inhibitory substance (Cate, et al., Cell, 45:685-698 (1986)), as well as the

predicted products of both a pattern gene in Drosophila (Padgett, et al., Nature, 325:81-84 (1987)), and an amphibian gene expressed in frog oocytes (Weeks and Melton, Cell, 51:861-867 (1987)). Most recently, three new proteins, called bone morphogenetic proteins (BMPs), have been added to the family.

A unifying feature of the biology of these polypeptides is their ability to regulate developmental processes. The inhibins and activins, as discussed further below, regulate reproductive functions and erythropoietic activity. The BMPs are thought to play a role in the formation of cartilage and bone *in vivo*. TGF-β itself (Kimelman and Kirschner, Cell, 51:869-877 (1987)) has been shown to augment the ability of fibroblast growth factor to induce mesoderm and plays a pivotal role in morphogenesis and organogenesis in mammalian embryos. In addition, like activin, TGF-β is reported to possess follicle-stimulating-hormone (FSH)-releasing activity. (Ying, et al., Biochem. Biophys. Res. Commun., 135:950-956 (1986).)

Furthermore, TGF-β has been shown to inhibit the growth of several human cancer cell lines (Roberts et al., PNAS, 82:119-123 (1985)). TGF-β has also been described as being able to inhibit production of the HIV virus and decrease syncytia formation, U.S. Pat. No. 5,236,905.

Activins have an extensive anatomical distribution and are implicated in the regulation of many biological processes, including the proliferation of many cell lines (Gonzalez-Manchon, C. and Vale, W., Endocrinology, 125:1666-1672 (1989)), control of the secretion and expression of the anterior pituitary hormones FSH, GH, and ACTH (Vale, W., et al., Nature, 321:776-779 (1986)), neuronal survival (Hashimoto, M., et al., Biochem. Biophys. Res. Commun., 173:193-200 (1990)), hypothalamic oxytocin secretion (Sawchenko, P.E., et al., Nature, 334:615-617 (1988)), erythropoiesis (Eto, Y., et al., Biochem. Biophys. Res.

Commun., 142:1095-1103 (1987)), and early embryonic development (Green and Smith, Nature, 347:391-394 (1990)). Activin has also been shown to suppress androgen production (Hsueh et al., PNAS, 84:5082-5086 (1987)), therefore activin decreases follicular size and may lead to female infertility (Woodruff et al., Endocrinol., 127:3196-3205 (1990)).

There are three activins (A, B, and AB), comprising different combinations of two closely-related beta sub-units, (Mason, A.J., et al., Nature, 318:659-663 (1985)). Activins impinge on a much broader spectrum of cells than inhibins, however, in those systems in which both proteins are functional, they have opposing biological effects. The mechanistic basis for this antagonism is unknown.

Cross-linking of labeled TGF-β to membrane receptors has revealed three distinct classes of integral cell membrane components that bind TGF-β (Massague, J., Biol. Chem., 260:7059-7066 (1985)). Class I components are 65 Kd in all species, whereas Class II components range from 85 Kd in rodent cells to 95 Kd in monkey and human cells to 110 Kd in chicken cells. Most frequently, all three classes of these binding proteins coexist on cells.

Type I and II TGF-β receptor components, like most growth factor receptors, are glycoproteins. The type III protein is a proteoglycan consisting predominantly of heparin sulfate glycosaminoglycan chains with a smaller amount of chondroitin or dermatan sulfate attached to a core protein of about 100-140 Kd. The binding site for TGF-β resides in this core protein (Cheifetz, et al., J. Biol. Chem., 263:16904-16991 (1988)).

An isolated TGF-β supergene family receptor polypeptide has been disclosed in U.S. Patent No. 5,216,126. However, this receptor polypeptide has approximately 75% sequence identity with the mature human inhibin/activin receptor sequence. A protein designated as an "activin receptor" has been expression-cloned (Mathews and Vale, Cell, 65:1-20

(1991)), however, it does not appear to be a mammalian receptor in that it is a serine kinase.

In accordance with one aspect of the present invention, there are provided novel polypeptides which have been identified as TAR-1 and TAR-3 receptors, as well as fragments, analogs and derivatives thereof. The receptor polypeptides of the present invention are of human origin.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such receptor polypeptides.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such receptor polypeptides by recombinant techniques.

In accordance with yet a further aspect of the present invention, there are provided processes for utilizing such receptor polypeptides, or polynucleotides encoding such receptor polypeptides for therapeutic purposes, for example, to determine the extent of the receptors in the cells of a patient, to measure all bindable forms of activin and TGF-β and to screen for receptor antagonists and/or agonists to the TAR-1 and TAR-3 receptors.

In accordance with another aspect of the present invention there is provided a process of using such antagonists to treat liver and lung fibrosis.

In accordance with another aspect of the present invention there is provided a process of using such agonists for inhibiting proliferation of HIV virus and inhibiting the growth of cancer cells.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such receptor polypeptides.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 is a cDNA sequence and corresponding deduced amino acid sequence of the TAR-1 receptor polypeptide. The signal sequence of the receptor polypeptide is the first 15 amino acids indicated and the transmembrane portion is underlined.

Figure 2 is the cDNA sequence and corresponding deduced amino acid sequence of the TAR-3 receptor polypeptide wherein the first 21 amino acids represent the signal sequence and the transmembrane portion is underlined. The standard one-letter abbreviation for amino acids is used throughout the figures.

Figure 3 illustrates the amino acid sequence homology between TAR-1 and TAR-3 and rat type I TGF-β receptor. Boxed areas indicate identical amino acids between the different sequences.

Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Accordingly, the sequences of Figure 1 and 2 are based on several sequencing runs and the sequencing accuracy is considered to be at least 97%.

The receptors of the present invention have been putatively identified as a TAR-1 and TAR-3 receptor. This identification has been made as a result of amino acid sequence homology.

In accordance with an aspect of the present invention, there are provided isolated nucleic acid (polynucleotides) which encode for the mature receptor polypeptides having the deduced amino acid sequence of Figure 1 and Figure 2 or for the mature receptor polypeptides encoded by the cDNA of the clone deposited as ATCC Deposit No. 75843 and ATCC Deposit No. 75842 for TAR-1 and TAR-3, respectively, which were deposited on July 27, 1994.

The polynucleotide encoding for TAR-1 was discovered in a cDNA library derived from a human adult spleen. It is structurally related to the TGF-β receptor family. It contains an open reading frame encoding a protein of 509 amino acid residues of which approximately the first 27 amino acids residues are the putative signal sequence such that the mature protein comprises 482 amino acids. TAR-1 exhibits the highest degree of homology to type I TGF-β receptor with 49% identity and 67% similarity over a 503 amino acid stretch.

The polynucleotide encoding for TAR-3 was discovered in a cDNA library derived from a human placenta. It is also structurally related to the TGF-β receptor family. It contains an open reading frame encoding a protein of about 503 amino acid residues of which approximately the first 21 amino acids residues are the putative signal sequence such that the mature protein comprises 482 amino acids. TAR-3 also exhibits the highest degree of homology to type I TGF-β receptor with 47% identity and 64% similarity over a 500 amino acid stretch.

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature receptor polypeptides may be identical to the coding sequence shown in Figure 1 and Figure 2 or that of the deposited clone(s) or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same, mature receptor polypeptides as the DNA of Figure 1 and Figure 2 or the deposited cDNA.

The polynucleotides which encode for the mature receptor polypeptides of Figure 1 and Figure 2 or for the mature receptor polypeptides encoded by the deposited cDNA(s) may include: only the coding sequence for the mature receptor

polypeptides; the coding sequence for the mature receptor polypeptides and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature receptor polypeptides (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature receptor polypeptides.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequence of Figure 1 and Figure 2 or the polypeptide encoded by the cDNA(s) of the deposited clone(s). The variant of the polynucleotides may be a naturally occurring allelic variant of the polynucleotides or a non-naturally occurring variant of the polynucleotides.

Thus, the present invention includes polynucleotides encoding the same mature receptor polypeptides as shown in Figure 1 and Figure 2 or the same mature receptor polypeptides encoded by the cDNA(s) of the deposited clone(s) as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the receptor polypeptides of Figure 1 and Figure 2 or the receptor polypeptides encoded by the cDNA(s) of the deposited clone(s). Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figure 1 and Figure 2 or of the coding sequences of the deposited clone(s). As known in the art, an allelic variant is an alternate form of

a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature receptor polypeptides may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotides of the present invention may encode for mature receptor proteins, or for a receptor protein having a prosequence or for a receptor protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the receptor polypeptides of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature receptor polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza

hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides . As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode receptor polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA(s) of Figure 1 and Figure 2 or the deposited cDNA(s).

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to TAR-1 and TAR-3 receptor polypeptides which have the deduced amino acid sequence of Figure 1 and Figure 2 or which have the amino acid sequences encoded by the deposited cDNA(s), as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the receptor polypeptides of Figure 1 and Figure 2 or that encoded by the deposited cDNA(s), means receptor polypeptides which retain essentially the same biological function or activity as such polypeptides. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce active mature receptor polypeptides.

The polypeptides of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the receptor polypeptides of Figure 1 and Figure 2 or that encoded by the deposited cDNA(s) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-

occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form cf a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the TAR-1 and TAR-3 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing receptor polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies.

However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the proteins.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The

selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast

cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the receptor polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter ennancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and

a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone"

sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The TAR-1 and TAR-3 receptor polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite

chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The receptor polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. The polypeptides may also include an initial methionine residue.

The present invention also relates to detecting altered levels of expression of mRNA encoding TAR-1 and TAR-3 receptors. The overexpression of these genes or loss of expression is indicative of cancer or the risk of cancer. Assays employed to detect the expression of these nucleic acid sequences are well-known by those skilled in the art and include, for example, nucleic acid probe hybridization, PCR and Southern blot analysis. In the hybridization technique, a sample is derived from a host and analyzed to determine whether the sample contains altered levels of TAR receptor gene expression.

TAR-1 and TAR-3 receptor polypeptides are useful in radio-receptor assays to measure all bindable (and active) forms of a TGF supergene family member such as activin. Such an assay would be specific for activin and would be conducted using the naturally purified or recombinant TAR-1 and TAR-3 receptors as the receptor element.

In addition, TAR-1 and TAR-3 receptor polypeptides are useful for screening for compounds that bind to them and have TGF supergene family biological activity as defined above. Preferably, these compounds are small molecules such as

organic or peptide molecules that exhibit one or more of the desired activities of a TGF supergene family member such as activin. Screening assays of this kind are conventional in the art, and any such screening procedure may be employed, whereby the test sample is contacted with the TAR-1 or TAR-3 receptor herein and the extent of binding and biological activity of the bound molecules is determined.

TAR-1 and TAR-3 receptor polypeptides are additionally useful in affinity purification of TGF supergene family members that bind TAR-1 and TAR-3 receptors and in purifying antibodies thereto. The receptor is typically coupled to an immobilized resin such as Affi-Gel 10 (Bio-Rad, Richmond, CA) or other such resins (support matrices) by means well-known in the art. The resin is equilibrated in a buffer and the preparation to be purified is placed in contact with the resin, whereby the molecules are selectively adsorbed to the receptor on the resin. The resin is then sequentially washed with suitable buffers to remove non-adsorbed material, including unwanted contaminants, from the mixture to be purified, using, for an activin mixture, e.g., 100mM glycine, pH 3, and 0.1% octyl β-glucoside. The resin is then treated so as to elute the compound using a buffer that will break the bond between the compound and receptor.

The TAR-1 and TAR-3 receptors of the present invention may also be employed in a process for screening for antagonists and/or agonists for the receptors. As an example, mammalian cells or membrane preparations expressing either the TAR-1 or TAR-3 receptor would be incubated with labeled ligand in the presence of compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of ligand and receptor would be measured compared in the presence or absence of the compound. Such second messenger systems include but are not

limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

Other screening techniques include the use of cells which express the receptors (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in Science, volume 246, pages 181-296 (October 1989). For example, potential agonists or antagonists may be contacted with a cell which expresses the receptors and a second messenger response, e.g. signal transduction or pH changes, may be measured to determine whether the potential agonist or antagonist is effective.

Another such screening technique involves introducing RNA encoding the receptors into xenopus oocytes to transiently express the receptor. The receptor oocytes may then be contacted in the case of antagonist screening with the receptor ligand and a compound to be screened, followed by detection of inhibition of a calcium signal.

Another screening technique involves expressing the TAR-1 or TAR-3 receptor in which the receptor is linked to a phospholipase C or D. As representative examples of such cells, there may be mentioned endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening for an antagonist or agonist may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase second signal.

A potential antagonist is an antibody, or in some cases an oligonucleotide, which binds to the TAR-1 and TAR-3 receptors but does not elicit a second messenger response such that the activity of the receptors is prevented.

Potential antagonists also include proteins which are closely related to the ligand of the receptors, i.e. a fragment of the ligand, which have lost biological function

and when binding to the TAR-1 and TAR-3 receptors, elicit no response.

A potential antagonist also includes an antisense construct prepared through the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of TAR-1 and TAR-3 receptors. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the receptors (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the receptors.

Another potential antagonist is a small molecule which binds to the TAR-1 and/or TAR-3 receptors, making them inaccessible to ligands such that normal biological activity is prevented. Examples of small molecules include but are not limited to small peptides or peptide-like molecules.

Potential antagonists also include a soluble form of a TAR-1 and TAR-3 receptors, e.g. a fragment of the receptor, which binds to the ligand and prevents the ligand from interacting with membrane bound receptors. These soluble

fragments bind to TGF-β and/or activin and prevent binding to membrane-bound receptors.

TGF-β stimulates fibroblasts to migrate to a site of injury, proliferate and produce collagen, TGF-β also inhibits collagen degradation. These excessive levels of collagen lead to liver and lung fibrosis. Accordingly, antagonists of TAR-1 and TAR-3 receptors will prevent liver and lung fibrosis.

The antagonists may also be employed to treat female sterility, since activin decreases follicle size and may lead to this disorder. The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

Agonists to the TAR-1 and TAR-3 receptors may be employed to activate these receptors and upregulate the functional application of TGF-β. Since TGF-β has been implicated in suppressing growth of cancerous cells, the activation of the TAR-1 and TAR-3 receptor may be employed to treat cancer. TGF-β also inhibits growth and proliferation of the HIV virus and accordingly stimulating the TAR-1 and TAR-3 receptors treats HIV infection.

Agonists which stimulate these receptors also will upregulate activins and stimulate spermatogonial proliferation to treat male infertility.

The agonists and antagonists of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the agonist or antagonist and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of

the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, the pharmaceutical compositions will be administered in an amount of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The TAR-1 and TAR-3 receptor polypeptides and agonists and antagonists which are polypeptides may also be employed in accordance with the invention by expression of the polypeptides *in vivo*, which is often referred to as "gene therapy".

For example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding the TAR-1 and TAR-3 polypeptides *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptides. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA(s). Computer analysis of the cDNA(s) is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA(s) libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA(s) clones to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA(s) as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA(s) or genomic sequence between affected and

unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA(s) precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-

hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for

particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Expression of Recombinant TAR-1 in COS cells

The expression of plasmid, TAR-1 HA is derived from a vector pcDNA(s)I/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA

fragment encoding the entire TAR-1 precursor and a HA tag fused in frame to its 3' end was cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding TAR-1, ATCC # 75843, was constructed by PCR on the original EST cloned using two primers: the 5' primer 5' GCGCGAAGCTTTACAATGGTAGATGG AGTGAT 3' contains a Hind III site followed by 21 nucleotides of TAR-1 coding sequence including the initiation codon; the 3' sequence 5' GCGCGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGG GTAACAGTCAGTTTTCAATTTGT 3' contains complementary sequences to an Xho site, translation stop codon, HA tag and the last 20 nucleotides of the TAR-1 coding sequence (not including the stop codon). Therefore, the PCR product contains a Hind III site, TAR-1 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an Xho site. The PCR amplified DNA fragment and the vector, pcDNA(s)I/Amp, were digested with Hind III and Xho I restriction enzyme and ligated. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant TAR-1, COS cells were transfected with the expression vector by DEAE-DEXTRAN

method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the TAR-1 HA protein was detected by radiolabelling and immunoprecipitation method. (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Example 2

Expression of Recombinant TAR-3 in COS cells

The expression of plasmid, TAR-3 HA is derived from a vector pcDNA(s)I/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire TAR-3 precursor and a HA tag fused in frame to its 3' end was cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding TAR-3, ATCC No. 75842, was constructed by PCR on the Full-length TAR-3 clone using two primers: the 5' primer is 5' GCGCGAAGCTTACCATGACC TTGGGCTCCCCCA 3' contains a Hind III site followed by 22 nucleotides of TAR-3 coding sequence starting from the initiation codon; the 3' sequence 5' GCGCGTCTAGATCAAGCGTAG TCTGGGACGTCGTATGGGTAGTGAATCACTTTGGGCTTCTC 3' contains complementary sequences to an Xba I site, translation stop codon, HA tag and the last 21 nucleotides of the TAR-3 coding sequence (not including the stop codon). Therefore, the PCR product contains a Hind III site, TAR-3 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an Xba I site. The PCR amplified DNA fragment and the vector, pcDNA(s)I/Amp, were digested with HInd III and Xba I restriction enzyme and ligated. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant TAR-3, COS cells were transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the TAR-3 HA protein was detected by radiolabelling and immunoprecipitation method. (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA

specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HE, ET AL.

(ii) TITLE OF INVENTION: TAR-1 and TAR-3

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN

(B) STREET: 6 BECKER FARM ROAD

(C) CITY: ROSELAND

(D) STATE: NEW JERSEY

(E) COUNTRY: USA

(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 INCH DISKETTE

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE: Submitted herewith

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FERRARO, GREGORY D.
(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-132

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 1539 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCTCTCCTGC GGTAATGAGG ACCACTGTGA AGGCCAGCAG TGCTTTTCCT CACTGAGCAT  180
CAACGATGGC TTCCACTGCT ACCAGAAAGG CTGCTTCCAG GTTTATGAGC AGGGAAAGAT  240
GACCTGTAAG ACCCCGCCGT CCCCTGGCCA AGCTGTGGAG TGCTGCCAAG GGGACTGGTG  300
TAACAGGAAC ATCACGGCCC AGCTGCCCAC TAAAGGAAAA TCCTTCCCTG GAACACAGAA  360
TTTCCACTTG GAGGTTGGCC TCATTATCCT CTCTGTAGTG TTCGCAGTAT GTCTTTTAGC  420
CTGCCTGCTG GGAGTTGCTC TCCGAAAATT TAAAAGGCGC AACCAAGAAC GCCTCAATCC  480
CCGAGACGTG GAGTATGGCA CTATCGAGGG GCTCATCACC ACCAATGTGG GAGACAGCAC  540
TTTAGCAGAT TTATTGGATC ATTCGTGTAC ATCAGGAAGT GGCTCTGGTC TTCCTTTTCT  600
GGTACAAAGA ACAGTGGCTC GCCAGATTAC ACTGTTGGAG TGTGTCGGGA AAGGCAGGTA  660
TGGTGAGGTG TGGAGGGGCA GCTGGCAAGG GGAAAATGTT GCCGTGAAGA TCCTCTCCTC  720
CCGTGATGAG AAGTCATGGT TCAGGGAAAC GGAATTGTAC AACACTGTCA TGCTGAGGCA  780
TGAAAATATC TTAGGTTTCA TTGCTTCAGA CATGACATCA AGACACTCCA GTACCCAGCT  840
GTGGTTAATT ACACATTATC ATGAAATGGG ATCGTTGTAC GACTATCTTC AGCTTACTAC  900
TCTGGATACA GTTAGCTGTC TTCGAATAGT GCTGTCCATA GCTAGTGGTC TTGCACATTT  960
GCACATAGAG ATATTTGGCA CCCAAGGGAA ACCAGCCATT GCCCATCGAG ATTTAAAGAG 1020
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CAAAAATACT CTGGTTAAGA AGAATGGACA GTGTTGCATA GCAGATTTGG GCCTGGCAGT 1080
CATGCATTCC CAGAGCACCA ATCAGCTTGA TGTGGGGAAC AATCCCCGTG TGGGCACCAA 1140
GCGCTACATG GCCCCCGAAG TTCTAGATGA AACCATCCAG GTGGATTGTT TCGATTCTTA 1200
TAAAAGGGTC GATATTTGGG CCTTTGGACT TGTTTTGTGG GAAGTGGCCA GGCGGATGGT 1260
GAGCAATGGT ATAGTGGAGG ATTACAAGCC ACCGTTCTAC GATGTGGTTC CCAATAACCC 1320
AAGTTTTGAA GATATGAGGA AGGTAGTCTG TGTGGATCAA CAAAGGCCAA ACATACCCAA 1380
CAGATGGTTC TCAGACCCGA CATTAACCTC TCTGGCCAAG CTAATGAAAG AATGCTGGTA 1440
TCAAAATCCA TCCGCAAGAC TCACAGCACT GCGTATCAAA AAGACTTTGA CCAAAATTGA 1500
TAATTCCCTC GACAAATTGA AAACTGACTG TTGACATTT                          1539
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 509 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

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-25                 -20                 -15
Leu Pro Ser Pro Ser Met Glu Asp Glu Lys Pro Lys Val Asn Pro
-10                  -5                   1
Lys Leu Tyr Met Cys Val Cys Glu Gly Leu Ser Cys Gly Asn Glu
5                   10                  15
Asp His Cys Glu Gly Gln Gln Cys Phe Ser Ser Leu Ser Ile Asn
20                  25                  30
Asp Gly Phe His Val Tyr Gln Lys Gly Cys Phe Gln Val Tyr Glu
35                  40                  45
Gln Gly Lys Met Thr Cys Lys Thr Pro Pro Ser Pro Gly Gln Ala
50                  55                  60
Val Glu Cys Cys Gln Gly Asp Trp Cys Asn Arg Asn Ile Thr Ala
65                  70                  75
Gln Leu Pro Thr Lys Gly Lys Ser Phe Pro Gly Thr Gln Asn Phe
80                  85                  90
His Leu Glu Val Gly Leu Ile Ile Leu Ser Val Val Phe Ala Val
95                 100                 105
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Cys Leu Leu Ala Cys Leu Leu Gly Val Ala Leu Arg Lys Phe Lys
 110                 115                 120
Arg Arg Asn Gln Glu Arg Leu Asn Pro Arg Asp Val Glu Tyr Gly
 125                 130                 135
Thr Ile Glu Gly Leu Ile Thr Thr Asn Val Gly Asp Ser Thr Leu
 140                 145                 150
Ala Asp Leu Leu Asp His Ser Cys Thr Ser Gly Ser Gly Ser Gly
 155                 160                 165
Leu Pro Phe Leu Val Gln Arg Thr Val Ala Arg Gln Ile Thr Leu
 170                 175                 180
Leu Glu Cys Val Gly Lys Gly Arg Tyr Gly Glu Val Trp Arg Gly
 185                 190                 195
Ser Trp Gln Gly Gly Asn Val Ala Val Lys Ile Leu Ser Ser Arg
 200                 205                 210
Asp Glu Lys Ser Trp Phe Arg Glu Thr Glu Leu Tyr Asn Thr Val
 215                 220                 225
Met Leu Arg His Glu Asn Ile Leu Gly Phe Ile Ala Ser Asp Met
 230                 235                 240
Thr Ser Arg His Ser Ser Thr Gln Leu Trp Leu Ile Thr His Tyr
 245                 250                 255
His Glu Met Gly Ser Leu Tyr Asp Tyr Leu Gln Leu Thr Thr Leu
 260                 265                 270
Asp Thr Val Ser Cys Leu Arg Ile Val Leu Ser Ile Ala Ser Gly
 275                 280                 285
Leu Ala His Leu His Ile Glu Ile Phe Gly Thr Gln Gly Lys Pro
 290                 295                 300
Ala Ile Ala His Arg Asp Leu Lys Ser Lys Asn Thr Leu Val Lys
 305                 310                 315
Lys Asn Gly Gln Cys Cys Ile Ala Asp Leu Gly Leu Ala Val Met
 320                 325                 330
His Ser Gln Ser Thr Asn Gln Leu Asp Val Gly Asn Asn Pro Arg
 335                 340                 345
Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp Gly Thr
 350                 355                 360
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Ile Gln Val Asp Cys Phe Asp Ser Tyr Lys Arg Val Asp Ile Trp
365 370 375
Ala Phe Gly Leu Val Leu Trp Glu Val Ala Arg Arg Met Val Ser
380 385 390
Asn Gly Ile Val Gly Asp Tyr Lys Pro Pro Phe Tyr Asp Val Val
395 400 405
Pro Asn Asn Pro Ser Phe Glu Asp Met Arg Lys Val Val Cys Val
410 415 420
Asp Gln Gln Arg Pro Asn Ile Pro Asn Arg Trp Phe Ser Asp Pro
425 430 435
Thr Leu Thr Ser Leu Ala Lys Leu Met Lys Glu Cys Trp Tyr Gln
440 445 450
Asn Pro Ser Ala Arg Leu Thr Ala Leu Arg Ile Lys Lys Thr Leu
455 460 465
Thr Lys Ile Asp Asn Ser Leu Asp Lys Leu Lys Thr Asp Cys
470 475 480

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 1596 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCACGAGGAG GGAGCCACGG CCAGCGGCTG TAACACTTCA TGGCTCTTAC TCCACCTCTC 60
TTGCTCCTCT CTGAAGGGAC CATGACCTTG GGCTCCCCCA GGAAAGGCCT TCTGATGCTG 120
CTGATGGCCT TGGTGACCCA GGGAGACCCT GTGAAGCCGT CTCGGGGCCC GCTGGTGACC 180
TGCACGTGTG AGAGCCCACA TTGCAAGGGG CCTACCTGCC GGGGGGCCTG GTGCACAGTA 240
GTGCTGGTGC GGGAGGAGGG GAGGCACCCC CAGGAACATC GGGGCTGCGG GAACTTGCAC 300
AGGGAGCTCT GCAGGGGGCG CCCCACCGAG TTCGTCAACC ACTACTGCTG CGACAACCAC 360
CTCTGCAACC ACAACGTGTC CCTGGTGCTG GAGGCCACCC AACCTCCTTC GGAGCAACCG 420
GGAACAGATG GCCAGCTGGC CCTGATCCTG GGCCCCGTGC TGGCCTTGCT GGCCCTGGTG 480

```
GCCCTGGGTG TCCTGGGCCT GTGGCATGTC CGACGGAGGC AGGAGAAGCA GCGTGACCTG  540
CACAGCGAGC TGGGAGAGTC CAGTCTCATC CTGAAAGCAT CTGAGCAGGA CGACAGCATG  600
TTGGGGGACC TCCTGGACAG TGACTGCACC ACAGGGAGTG GCTCAGGGCT CCCCTTCCTG  660
GTGCAGAGGA CAGTGGCACG GCAGGTTGCC TTGGTGGAGT GTGTGGGAAA AGGCCGCTAT  720
GGCGAAGTGT GGCGGGGCTT GTGGCACGGT GAGAGTGTGG CCGTCAAGAT CTTCTCCTCG  780
AGGGATGAAC AGTCCTGGTT CCGGGAGACT GAGATCTATA ACACAGTGTT GCTCAGACAC  840
GACAACATCC TAGGCTTCAT CGCCTCAGAC ATGACCTCCC GCAACTCGAG CACGCAGCTG  900
TGGCTCATCA CGCACTACCA CGAGCACGGC TCCCTCTACG ACTTTCTGCG GAGACAGACG  960
CTGGAGCCCC ATCTGGCTCT GAGGCTAGCT GTGTCCGCGG CATGCGGCCT GGCGCACCTG 1020
CACGTGGAGA TCTTTGGTAC CACAGGGCAA CCAGCCATTG CCCACCGCGA CTTCAAGAAC 1080
CGCAATGTGC TGGTCAAGAG CAACCTGCAG TGTTGCATCG CCGACCTGGG CCTGGCTGTG 1140
ATGCACTCAC AGGGCAGCGA TTACCTGGAC ATCGGCAACA ACCCGAGAGT GGGCACCAAG 1200
CGGTACATGG CACCCGAGGT GCTGGACGAG CAGATCCGCA CGGACTGCTT TGAGTCCTAC 1260
AAGTGGACTG ACATCTGGGC CTTTGGCCTG GTGCTGTGGG AGATTGCCCG CCGGACCATC 1320
GTGAATGGCA TCGTGGAGGA CTATAGACCA CCCTTCTATG GTGTGGTGCC CAATGACCCC 1380
AGCTTTGAGG ACATGAAGAA GGTGGTGTGT GTGGATCAGC AGACCCCCAC CATCCCTAAC 1440
CGGCTGGCTG CAGACCCGGT CCTCTCAGGC CTAGCTCAGA TGATGCGGGA GTGCTGGTAC 1500
CCAAACCCCT CTGCCCGACT CAACGCGCTG CGGATCAAGA AGACACTACA AAAAATTAGT 1560
AACAGTCCAG AGAAGCCCAA AGTGATTCAC TAGCCC                            1596
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 503 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Thr Leu Gly Ser Pro Arg Lys Gly Leu Leu Met Leu Leu Met
    -20                 -15                 -10
Ala Leu Val Thr Gln Gly Asp Pro Val Lys Pro Ser Arg Gly Pro
     -5                   1                   5
Leu Val Thr Cys Thr Cys Glu Ser Pro His Cys Lys Gly Pro Thr
 10                  15                  20
Cys Arg Gly Ala Trp Cys Thr Val Val Leu Val Arg Glu Glu Gly
 25                  30                  35
```

```
Arg His Pro Gln Glu His Arg Gly Cys Gly Asn Leu His Arg Glu
 40                  45                  50
Leu Cys Arg Gly Arg Pro Thr Glu Phe Val Asn His Tyr Cys Cys
 55                  60                  65
Asp Asn His Leu Cys Asn His Asn Val Ser Leu Val Leu Glu Ala
 70                  75                  80
Thr Gln Pro Pro Ser Glu Gln Pro Gly Thr Asp Gly Gln Leu Ala
 85                  90                  95
Leu Ile Leu Gly Pro Val Leu Ala Leu Leu Ala Leu Val Ala Leu
100                 105                 110
Gly Val Leu Gly Leu Trp His Val Arg Arg Arg Gln Glu Lys Gln
115                 120                 125
Arg Asp Leu His Ser Glu Leu Gly Glu Ser Ser Leu Ile Leu Lys
130                 135                 140
Ala Ser Glu Gln Asp Asp Ser Met Leu Gly Asp Leu Leu Asp Ser
145                 150                 155
Asp Cys Thr Thr Gly Ser Gly Ser Gly Leu Pro Phe Leu Val Gln
160                 165                 170
Arg Thr Val Ala Arg Gln Val Ala Leu Val Glu Cys Val Gly Lys
175                 180                 185
Gly Arg Tyr Gly Glu Val Trp Arg Gly Leu Trp His Gly Glu Ser
190                 195                 200
Val Ala Val Lys Ile Phe Ser Ser Arg Asp Glu Gln Ser Trp Phe
205                 210                 215
Arg Glu Thr Glu Ile Tyr Asn Thr Val Leu Leu Arg His Asp Asn
220                 225                 230
Ile Leu Gly Phe Ile Ala Ser Asp Met Thr Ser Arg Asn Ser Ser
235                 240                 245
Thr Gln Leu Trp Leu Ile Thr His Tyr His Glu His Gly Ser Leu
250                 255                 260
Tyr Asp Phe Leu Arg Arg Gln Thr Leu Glu Pro His Leu Ala Leu
265                 270                 275
Arg Leu Ala Val Ser Ala Ala Cys Gly Leu Ala His Leu His Val
280                 285                 290
```

Glu Ile Phe Gly Thr Gln Gly Lys Pro Ala Ile Ala His Arg Asp
295 300 305
Phe Lys Asn Arg Asn Val Leu Val Lys Ser Asn Leu Gln Cys Cys
310 315 320
Ile Ala Asp Leu Gly Leu Ala Val Met His Ser Gln Gly Ser Asp
325 330 335
Tyr Leu Asp Ile Gly Asn Asn Pro Arg Val Gly Thr Lys Arg Tyr
340 345 350
Met Ala Pro Glu Val Leu Asp Glu Gln Ile Arg Thr Asp Cys Phe
355 360 365
Glu Ser Tyr Lys Trp Thr Asp Ile Trp Ala Phe Gly Leu Val Leu
370 375 380
Trp Glu Ile Ala Arg Arg Thr Ile Val Asn Gly Ile Val Glu Asp
385 390 395
Tyr Arg Pro Pro Phe Tyr Gly Val Val Pro Asn Asp Pro Ser Phe
400 405 410
Glu Asp Met Lys Lys Val Val Cys Val Asp Gln Gln Thr Pro Thr
415 420 425
Ile Pro Asn Arg Leu Ala Ala Asp Pro Val Leu Ser Gly Leu Ala
430 435 440
Gln Met Met Arg Glu Cys Trp Tyr Pro Asn Pro Ser Ala Arg Leu
445 450 455
Asn Ala Leu Arg Ile Lys Lys Thr Leu Gln Lys Ile Ser Asn Ser
460 465 470
Pro Glu Lys Pro Lys Val Ile His
475 480

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the groups consisting of:

(a) a polynucleotide encoding for the TAR-1 polypeptide having the deduced amino acid sequence of Figure 1 or a fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding for the TAR-3 polypeptide having the deduced amino acid sequence of Figure 2 or a fragment, analog or derivative of said polypeptide;

(c) a polynucleotide encoding for the TAR-1 polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75843 or a fragment, analog or derivative of said polypeptide;

(d) a polynucleotide encoding for the TAR-3 polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75842 or a fragment, analog or derivative of said polypeptide.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein said polynucleotide encodes for TAR-1 having the deduced amino acid sequence of Figure 1.

6. The polynucleotide of Claim 2 wherein said polynucleotide encodes for TAR-3 having the deduced amino acid sequence of Figure 1.

7. The polynucleotide of Claim 2 wherein said polynucleotide encodes for the TAR-1 polypeptide encoded by the cDNA(s) of ATCC Deposit No. 75843.

8. The polynucleotide of Claim 2 wherein said polynucleotide encodes for the TAR-3 polypeptide encoded by the cDNA(s) of ATCC Deposit No. 75842.

9. The polynucleotide of Claim 1 having the coding sequence for TAR-1 as shown in Figure 1.

10. The polynucleotide of Claim 1 having the coding sequence for TAR-3 as shown in Figure 2.

11. The polynucleotide of Claim 2 having the coding sequence for TAR-1 deposited as ATCC Deposit No. 75843.

12. The polynucleotide of Claim 2 having the coding sequence for TAR-3 deposited as ATCC Deposit No. 75843.

13. A vector containing the DNA of Claim 2.

14. A host cell genetically engineered with the vector of Claim 13.

15. A process for producing a polypeptide comprising: expressing from the host cell of Claim 14 the polypeptide encoded by said DNA.

16. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 13.

17. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having TAR-1 activity.

18. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having TAR-3 activity.

19. A polypeptide selected from the group consisting of (i) a TAR-1 polypeptide having the deduced amino acid sequence of Figure 1 and fragments, analogs and derivatives thereof and (ii) a TAR-3 polypeptide having the deduced amino acid sequence of Figure 2 and fragments, analogs and derivatives thereof, (iii) a TAR-1 polypeptide encoded by the cDNA of ATCC Deposit No. 75843 and fragments, analogs and derivatives of said polypeptide; and (iv) a TAR-3 polypeptide encoded by the cDNA of ATCC Deposit No. 75842 and fragments, analogs and derivatives of said polypeptide.

20. The polypeptide of Claim 19 wherein the polypeptide is TAR-1 having the deduced amino acid sequence of Figure 1.

21. The polypeptide of Claim 19 wherein the polypeptide is TAR-3 having the deduced amino acid sequence of Figure 2.

22. An antibody against the polypeptide of Claim 19.

23. A compound which inhibits activation of the polypeptide of Claim 19.

24. A compound which activates the polypeptide of claim 19.

25. A method for the treatment of a patient having need to activate TAR-1 comprising: administering to the patient a therapeutically effective amount of the compound of Claim 24.

26. A method for the treatment of a patient having need to activate TAR-3 comprising: administering to the patient a therapeutically effective amount of the compound of Claim 24.

27. A method for the treatment of a patient having need to inhibit TAR-1 comprising: administering to the patient a therapeutically effective amount of the compound of Claim 23.

28. A method for the treatment of a patient having need to inhibit TAR-3 comprising: administering to the patient a therapeutically effective amount of the compound of Claim 23.

29. The polypeptide of Claim 19 wherein the polypeptide is a soluble fragment of the TAR-1 receptor and is capable of binding a ligand for the receptor.

30. The polypeptide of Claim 19 wherein the polypeptide is a soluble fragment of the TAR-3 receptor and is capable of binding a ligand for the receptor.

31. A process for identifying antagonists and agonists to a TAR receptor comprising:

preparing cells for expression of the receptor;

contacting the cell with a receptor ligand and a compound to be screened;

determining the signal generated by the cell in response to binding of the ligand; and

identifying antagonists or agonists to the receptor.

32. A process for detecting cancer or the susceptibility to cancer by determining in a sample derived from a host the level of TAR-1 and TAR-3 receptors, whereby an elevated level indicates a cancer or the susceptibility to cancer.

FIG. 1A

```
1    TACAATGGTAGATGGAGTGATGATTCTTCCTGTGCTTATC
1                M  V  D  G  V  M  I  L  P  V  L  I
41   ATGATTGCTCTCCCCTCCCCTAGTATGGAAGATGAGAAGGCCCAAGGTCAACCCCAAACTC
13   M  I  A  L  P  S  P  S  M  E  D  E  K  P  K  V  N  P  K  L
101  TACATGTGTGTGTGAAGGTCTCTCCTGCGGTAATGAGGACCACTGTGAAGGCCAGCAG
33   Y  M  C  V  C  E  G  L  S  C  G  N  E  D  H  C  E  G  Q  Q
161  TGCTTTTCCTCACTGAGCATCAACGATGGCTTCCACGTCTACCAGAAAGGCTGCTTCCAG
53   C  F  S  S  L  S  I  N  D  G  F  H  V  Y  Q  K  G  C  F  Q
221  GTTTATGAGCAGGGAAAGATGACCTGTAAGACCCCGCCGTCCCCTGGCCAAGCTGTGGAG
73   V  Y  E  Q  G  K  M  T  C  K  T  P  P  S  P  G  Q  A  V  E
281  TGCTGCCAAGGGGACTGGTGTAACAGGAACATCACGGCCCAGCTGCCCACTAAAGGAAAA
93   C  C  Q  G  D  W  C  N  R  N  I  T  A  Q  L  P  T  K  G  K
341  TCCTTCCCTGGAACACAGAATTTCCACTTGGAGGTTGGCCTCATTATCCTCTCTGTAGTG
113  S  F  P  G  T  Q  N  F  H  L  E  V  G  L  I  I  L  S  V  V
401  TTCGCAGTATGTCTTTTAGCCTGCTGGGAGTTGCTCTCCGAAAATTTAAAAGGCGC
133  F  A  V  C  L  L  A  C  L  L  G  V  A  L  R  K  F  K  R  R
461  AACCAAGAACGCCTCAATCCCCGAGACGTGGAGTATGGCACTATCGAGGGGCTCATCACC
153  N  Q  E  R  L  N  P  R  D  V  E  Y  G  T  I  E  G  L  I  T
521  ACCAATGTGGGAGACAGCACTTTAGCAGATTTATTGGATCATTCGTGTACATCAGGAAGT
173  T  N  V  G  D  S  T  L  A  D  L  L  D  H  S  C  T  S  G  S
581  GGCTCTGGTCTTCCTTTCTGGTACAAAGAACAGTGGCTCGCCAGATTACACTGTTGGAG
193  G  S  G  L  P  F  L  V  Q  R  T  V  A  R  Q  I  T  L  L  E
641  TGTGTCGGGAAAGGCAGGTATGGTGAGGTGTGGAGGGGCAGCTGGCAAGGGGAAAATGTT
213  C  V  G  K  G  R  Y  G  E  V  W  R  G  S  W  Q  G  E  N  V
701  GCCGTGAAGATCCCTCTCCCGTGATGAGAAGTCATGGTTCAGGGAAACGGAATTGTAC
233  A  V  K  I  L  S  S  R  D  E  K  S  W  F  R  E  T  E  L  Y
761  AACACTGTGATGCTGAGGCATGAAATATCTTAGGTTTCATTGCTTCAGACATGACATCA
```

MATCH WITH FIG. 1B

MATCH WITH FIG. 1A

FIG. 1B

```
 253      N  T  V  M  L  R  H  E  N  I  L  G  F  I  A  S  D  M  T  S
 821      AGACACTCCAGTACCCAGCTGTGGTTAATTACACATTATCATGAAATGGGATCGTTGTAC
 273      R  H  S  S  T  Q  L  W  L  I  T  H  Y  H  E  M  G  S  L  Y
 881      GACTATCTTCAGCTTACTACTCTGGATACAGTTAGCTGTCTTCGAATAGTGCTGTCCATA
 293      D  Y  L  Q  L  T  T  L  D  T  V  S  C  L  R  I  V  L  S  I
 941      GCTAGTGGTCTTGCACATTTGCACATAGAGATATTTGGCACCCAAGGGAAACCAGCCATT
 313      A  S  G  L  A  H  L  H  I  E  I  F  G  T  Q  G  K  P  A  I
1001      GCCCATCGAGATTTAAAGAGCAAAAATACTCTGGTTAAGAAGAATGGACAGTGTTGCATA
 333      A  H  R  D  L  K  S  K  N  T  L  V  K  K  N  G  Q  C  C  I
1061      GCAGATTTGGGCCTGGCAGTCATGCATTCCCAGAGCACCAATCAGCTTGATGTGGGGAAC
 353      A  D  L  G  L  A  V  M  H  S  Q  S  T  N  Q  L  D  V  G  N
1121      AATCCCCGTGTGGGCACCAAGCGCTACATGGCCCCCGAAGTTCTAGATGAAACCATCCAG
 373      N  P  R  V  G  T  K  R  Y  M  A  P  E  V  L  D  E  T  I  Q
1181      GTGGATTGTTTCGATTCTTATAAAAGGGTCGATATTTGGGCCTTTGGACTTGTTTTGTGG
 393      V  D  C  F  D  S  Y  K  R  V  D  I  W  A  F  G  L  V  L  W
1241      GAAGTGGCCAGGCGGATGGTGAGCAATGGTATAGTGGAGGATTACAAGCCACCGTTCTAC
 413      E  V  A  R  R  M  V  S  N  G  I  V  E  D  Y  K  P  P  F  Y
1301      GATGTGGTTCCCAATAACCCAAGTTTTGAAGATATGAGGAAGGTAGTCTGTGTGGATCAA
 433      D  V  V  P  N  N  P  S  F  E  D  M  R  K  V  V  C  V  D  Q
1361      CAAAGGCCAAACATACCCAACAGATGGTTCTCAGACCCGACATTAACCTCTCTGGCCAAG
 453      Q  R  P  N  I  P  N  R  W  F  S  D  P  T  L  T  S  L  A  K
1421      CTAATGAAAGAATGCTGGTATCAAAATCCATCCGCAAGACTCACAGCACTGCGTATCAAA
 473      L  M  K  E  C  W  Y  Q  N  P  S  A  R  L  T  A  L  R  I  K
1481      AAGACTTTGACCAAAATTGATAATTCCCTCGACAAATTGAAAACTGACTGTTGACATTT
          K  T  L  T  K  I  D  N  S  L  D  K  L  K  T  D  C  *
```

SUBSTITUTE SHEET (RULE 26)

FIG. 2A

```
1    GCACGAGGAGGGAGCCACGGCCAGCGGCTGTaACACTTCATGGCTCTTACTCCACCTCTC
61   TTGCTCCTCTCTGAAGGGACCATGACCTTGGGCTCCCCCAGGAAAGGCCTTCTGATGCTG
1                 M  T  L  G  S  P  R  K  G  L  L  M  L
121  CTGATGGcCTTGGTGACCCAGGGAGACCCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACC
14   L  M  A  L  V  T  Q  G  D  P  V  K  P  S  R  G  P  L  V  T
181  TGCACGTGTGAGAGCCCACATTGCAAGGGGCCTACCTGCCGGGGGGCCTGGTGCACAGTA
34   C  T  C  E  S  P  H  C  K  G  P  T  C  R  G  A  W  C  T  V
241  GTGCTGGTGCGGGAGGAGGGGAGGCACCCCCAGGAACATCGGGGCTGCGGGAACTTGCAC
54   V  L  V  R  E  E  G  R  H  P  Q  E  H  R  G  C  G  N  L  H
301  AGGGAGCTCTGCAGGGGGCGCCCCACCGAGTTCGTCAACCACTACTGCTGCGACAACCAC
74   R  E  L  C  R  G  R  P  T  E  F  V  N  H  Y  C  C  D  N  H
361  CTCTGCAACCACACAACGTGTCCCTGGTGCTGGAGGCCACCCAACCTCCTTCGGAGCAACCG
94   L  C  N  H  N  V  S  L  V  L  E  A  T  Q  P  P  S  E  Q  P
421  GGAACAGATGGCCAGCTGGCCCTGATCCTGGGCCCCGTGCTGGCCTTGCTGGCCCTGGTG
114  G  T  D  G  Q  L  A  L  I  L  G  P  V  L  A  L  L  A  L  V
481  GCCCTGGGTGTCCTGGGCCTGTGGCATGTCCGACGGAGGCAGGAAGCAGCGTGACCTG
134  A  L  G  V  L  G  L  W  H  V  R  R  R  Q  E  K  Q  R  D  L
541  CACAGCGAGCTGGGAGAGTCCAGTCTCATCCTGAAAGCATCTGAGCAGGACGACAGCATG
154  H  S  E  L  G  E  S  S  L  I  L  K  A  S  E  Q  D  D  S  M
601  TTGGGGGACCTCCTGGACAGTGACTGCACCACAGGGAGTGGCTCAGGGCTCCCCTTCCTG
174  L  G  D  L  L  D  S  D  C  T  T  G  S  G  G  L  P  F  L
661  GTGCAGAGGACAGTGGCACGGCAGGTTGCCTTGGTGGAGTGTGTGGGAAAAGGCCGCTAT
194  V  Q  R  T  V  A  R  Q  V  A  L  V  E  C  V  G  K  G  R  Y
721  GGCGAAGTGTGGCGGGGCTTGTGGCACGGTGAGAGTGTGGCCGTCAAGATCTTCTCCTCG
214  G  E  V  W  R  G  L  W  H  G  E  S  V  A  V  K  I  F  S  S
781  AGGGATGAACAGTCCTGGTTCCGGGAGACTGAGATCTATAACACAGTGTTGCTCAGACAC
234  R  D  E  Q  S  W  F  R  E  T  E  I  Y  N  T  V  L  L  R  H
841  GACAACATCCTAGGCTTCATCGCCTCAGACATGACCTCCCGCAACTCGAGCACGCAGCTG
     MATCH WITH FIG. 2B
```

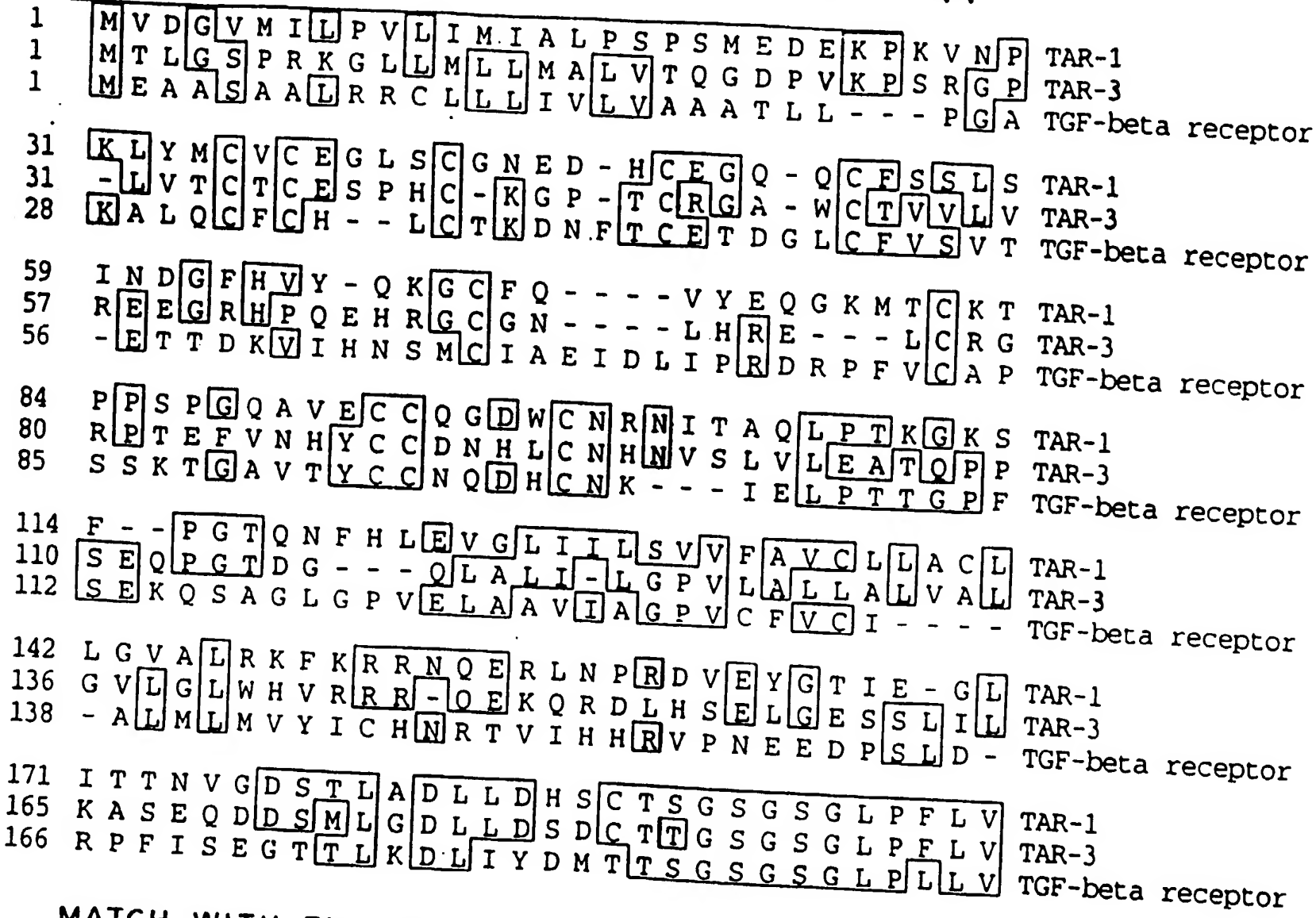
MATCH WITH FIG. 2A

FIG. 2B

```
254  D N I L G F I A S D M T S R N S S T Q L
901  TGGCTCATCACGCACTACCACGAGCACGGCTCCCTCTACGACTTTCTGCGGAGACAGACG
274  W L I T H Y H E H G S L Y D F L R R R Q T
961  CTGGAGCCCCCATCTGGCTCTGAGGCTAGCTGTGTCCGCGGCATGCGGCCTGGCGCACCTG
294  L E P H L A L R L A V S A A C G L A H H L
1021 CACGTGGAGATCTTTGGTACACAGGGCAAACCAGCCATTGCCCACCGCGACTTCAAGAAC
314  H V E I F G T Q G G K P A I A H R D F K N
1081 CGCAATGTGCTGGTCAAGAGCAACCTGCAGTGTTGCATCGCCGACCTGGGCCTGGCTGTG
334  R N V L V K S N L Q C C I A D L G L A V
1141 ATGCACTCACAGGGCAGCGATTACCTGGACATCGGCAACACCCGAGAGTGGGCACCAAG
354  M H S Q G S D Y L D I G N N P R V G T K
1201 CGGTACATGGCACCCGAGGTGCTGGACGAGCAGATCCGCACGGACTGCTTTGAGTCCTAC
374  R Y M A P E V L D E Q I R T D C F E S Y
1261 AAGTGGACTGACATCTGGGCCTTTGGCCTGGTGCTGTGGGAGATTGCCCGCCGGACCATC
394  K W T D I W A F G L V L W E I A R R T I
1321 GTGAATGGCATCGTGGAGGACTATAGACCACCCTTCTATGGTGTGGTGCCCAATGACCCC
414  V N G I V E D Y R P P F Y G V V P N D P
1381 AGCTTTGAGGACATGAAGAAGGTGGTGTGTGGATCAGCAGACCCCCACCATCCCTAAC
434  S F E D M K K V V C V D Q Q T P T I P N
1441 CGGCTGGCTGCAGACCCGGTCCTCTCAGGCCTAGCTCAGATGATGCGGGAGTGCTGGTAC
454  R L A A D P V L S G L A Q M M R E C W Y
1501 CCAAACCCCTCTGCCCGACTCAACGCGCTGCGGATCAAGAAGACACTACAAAAAATTAGT
474  P N P S A R L N A L R I K K T L Q K I S
1561 AACAGTCCAGAGAAGCCCAAAGTGATTCACTAGCCC
494  N S P E K P K V I H *
```

SUBSTITUTE SHEET (RULE 26)

FIG. 3A



MATCH WITH FIG. 3B

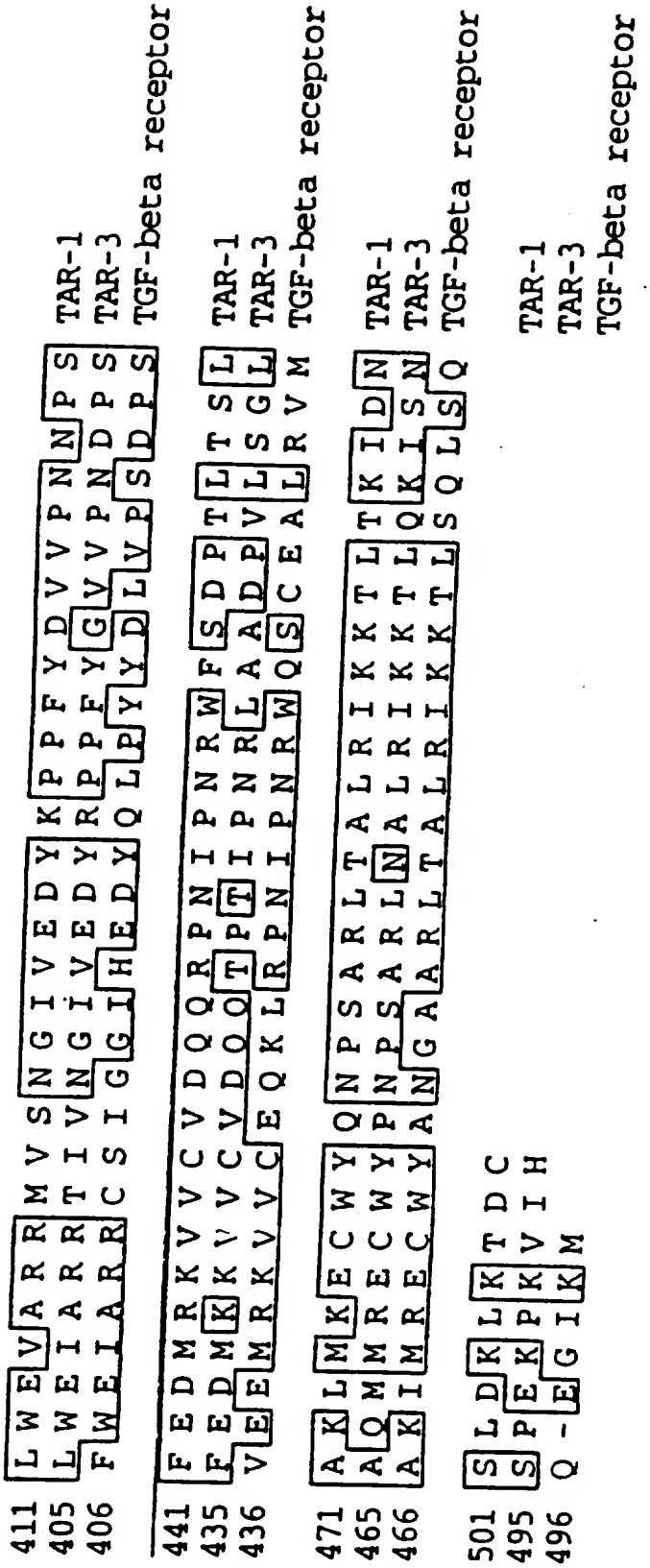
FIG. 3B

MATCH WITH FIG. 3A

```
201 QRTVARQITLECVGKGRYGEVWRGSWQGE  TAR-1
195 QRTVARQVALVECVGKGRYGEVWRGLWHGE  TAR-3
196 QRTIARTIVLQESIGKGRFGEVWRGKWRGE  TGF-beta receptor

231 NVAVKILSSRDEKSWFRETELYNTVMLRHE  TAR-1
225 SVAVKIFSSRDEQSWFRETEIYNTVLLRHD  TAR-3
226 EVAVKIFSSREERSWFREAEIYQTVMLRHE  TGF-beta receptor

261 NILGFIASDMTSRHSSTQLWLITHYHEMGS  TAR-1
255 NILGFIASDMTSRNSSTQLWLITHYHEHGS  TAR-3
256 NILGFIADNKDNGTWTQLWLVSDYHEHGS  TGF-beta receptor

291 LYDYLQTTLDTVSCLRIVLSIASGLAHLH  TAR-1
285 LYDELRKQTLEPHLALRLAVSAACGLAHLH  TAR-3
286 LFDYLNRYTVTVEGMIKLALSTASGLAHLH  TGF-beta receptor

321 IEIFGTQGKPAIAHRDLKSKNTLVKKNGQC  TAR-1
315 VEIFGTQGKPAIAHRDEKNRNVLVKSNLQC  TAR-3
316 MEIVGTQGKPAIAHRDLKSKNILVKKNGTC  TGF-beta receptor

351 CIADLGLAVMHSQSTNQLDVGNNPRVGTKR  TAR-1
345 CIADLGLAVMHSQGSDYLDIGNNPRVGTKR  TAR-3
346 CIADLGLAVRHDSATDTIDIAPNHRVGTKR  TGF-beta receptor

381 YMAPEVLDETIQVDCFDSYKRVDIWAFGLV  TAR-1
375 YMAPEVLDEQIRTDCFESYKWTDIWAFGLV  TAR-3
376 YMAPEVLDDSINMKHFESFKRADIYAMGLV  TGF-beta receptor
```

MATCH WITH FIG. 3C

FIG. 3C


MATCH WITH FIG.3B
411 L W E V A R R M V S N G I V E D Y K P P F Y D V V P N N P S TAR-1
405 L W E I A R R T I V N G I V E D Y R P P F Y G V V P N D P S TAR-3
406 F W E I A R R C S I G G I H E D Y Q L P Y Y D L V P S D P S TGF-beta receptor
441 F E D M R K V V C V D Q Q R P N I P N R W F S D P T L T S L TAR-1
435 F E D M K K V V C V D Q Q T P T I P N R L A A D P V L S G L TAR-3
436 V E E M R K V V C E Q K L R P N I P N R W Q S C E A L R V M TGF-beta receptor
471 A K L M K E C W Y Q N P S A R L T A L R I K K T L T K I D N TAR-1
465 A Q M M R E C W Y P N P S A R L N A L R I K K T L Q K I S N TAR-3
466 A K I M R E C W Y A N G A R L T A L R I K K T L S Q L S Q TGF-beta receptor
501 S L D K L K T D C TAR-1
495 S P E K P K V I H TAR-3
496 Q - E G I K M TGF-beta receptor

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11328

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 70.1, 70.3, 71.1, 172.1, 172.3, 240.2, 320.1; 530/300, 350; 536/23.1, 23.5, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Sequence Databases: EMBL, GenBank

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; STN files: Biosis, Medline, EMBASE, CA, WPIDS
search terms: activin; activin(with)receptor(s)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Oncogene, Vol. 8, Number 10, issued 1993, P. ten Dijke et al., "Activin Receptor-like Kinases: A Novel Subclass of Cell-surface Receptors with Predicted Serine/Threonine Kinase Activity", pages 2879-2887, see entire document, especially pages 2881-2882.	1-21, 29-30
Y	Cell, Vol. 75, issued 19 November 1993, L. Attisano et al., "Identification of Human Activin and Tgfβ Type I Receptors That Form Heteromeric Kinase Complexes with Type II Receptors", pages 671-680, see entire document.	1-21, 29-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
12 FEBRUARY 1995

Date of mailing of the international search report
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Int. ..ional application No.
PCT/US94/11328

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proc. Natl. Acad. Sci. USA, Vol. 90, issued December 1993, Tsuchida et al., "Cloning and Characterization of a Transmembrane Serine Kinase That Acts as an Activin Type I Receptor", pages 11242-11246, especially page 11243, Fig. 1.	1-21, 29-30
Y	Cell, Vol. 68, issued 10 January 1992, L. Attisano et al., "Novel Activin Receptors: Distinct Genes and Alternative mRNA Splicing Generate a Repertoire of Serine/Threonine Kinase Receptors", pages 97-108, see entire document.	1-21, 29-30
Y	Science, Vol. 260, issued 28 May 1993, R. Ebner et al., "Cloning of a Type I TGF-β Receptor and Its Effect on TGF-β Binding to the Type II Receptor", pages 1344-1348, see entire document.	1-21, 29-30
A	Cell, Vol. 68, issued 21 February 1992, H. Lin, et al., "Expression Cloning of the TGF-β Type II Receptor, a Functional Transmembrane Serine/Threonine Kinase", pages 775-785, see entire document.	1-21, 29-30
Y	Cell, Vol. 65, issued 14 June 1991, L. Mathews et al., "Expression Cloning of an Activin Receptor, a Predicted Transmembrane Serine Kinase", pages 973-982, see entire document.	1-21, 29-30
Y	Science, Vol. 255, issued 27 March 1992, L. Mathews et al., "Cloning of a Second Type of Activin Receptor and Functional Characterization in *Xenopus* Embryos", pages 1702-1705, see entire document.	1-21, 29-30
Y	WO, A, 92/20793 (MATHEWS ET AL.) 26 November 1992, see entire document.	1-21, 29-30
Y	WO, A, 93/19177 (DONAHOE ET AL.) 30 September 1993, see entire document.	1-21, 29-30
Y	Developmental Dynamics, Vol. 196, issued 1993, W. He et al., "Developmental Expression of Four Novel Serine.Threonine Kinase Receptors Homologous to the Activin/Transforming Growth Factor-β Type II Receptor Family", pages 133-142, see entire document.	1-21, 29-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11328

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Biological Chemistry, Vol. 268, No. 17, issued 15 June 1993, K. Matsuzaki et al., "A Widely Expressed Transmembrane Serine/Threonine Kinase That Does Not Bind Activin, Inhibin, Transforming Growth Factor β, or Bone Morphogenic Factor", pages 12719-12723, see entire document.	1-21, 29-30

INTERNATIONAL SEARCH REPORT

Int. ional application No.
PCT/US94/11328

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-21, 29-30

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/US94/11328

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12N 5/00, 5/10, 5/16, 7/00, 7/01, 15/00, 15/09, 15/10, 15/11, 15/12, 15/63; C12P 21/00, 21/02; C07K 2/00, 14/00, 14/705, 14/71, 14/715; C07H 21/00, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 70.1, 70.3, 71.1, 172.1, 172.3, 240.2, 320.1; 530/300, 350; 536/23.1, 23.5, 24.31

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-21 and 29-30, drawn to polynucleotides and polypeptides, classified in U.S. Class 536, subclass 23.1; Class 435, subclass 69.1; Class 435, subclass 320.1; Class 530, subclass 350; Class 530, subclass 300.

Group II, claim 22, drawn to an antibody, classified in U.S. Class 530, subclass 389.1; Class 530, subclass 388.22.

Group III, claims 23-24, drawn to compounds, classification dependent on species.

Group IV, claims 25-28, drawn to methods of treatment, classification dependent on species.

Group V, claim 31, drawn to process for identifying antagonists and agonists, classified in U.S. Class 436, subclass 501.

Group VI, claim 32, drawn to process for detecting cancer or susceptibility to cancer, classification dependent on species employed in said process.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I and II are related insofar as the polypeptides of claim 19 can be used as the antigens with which to generate the antibody of claim 22. However, the groups are distinct because the polypeptides of claim 19 can be used for a materially different purpose, such as to screen for ligands that bind to the polypeptides.

Groups I and III are related insofar as the polypetides of group I can be used to screen for agonists and antagonists of the polypeptides. However, the groups are distinct because the polypeptides of group I can be used for a materially different purpose, such as for generating antibodies against the polypeptides for diagnostic, research or therapeutic purposes.

Groups I and IV are related only insofar as the compound of group IV can potentially be used therapeutically by virtue of their ability to bind the polypeptides of group I. However, the groups are distinct because the polypeptides of group I can be used for a materially different purpose, such as for generating antibodies against the polypeptides for diagnostic, research or therapeutic purposes.

Groups I and V are related insofar as the polypeptides of group I can be used to identify agonists or antagonists of the claimed receptors. However, the groups are distinct because the polypeptides of group I can be used for a materially different purpose, such as for generating antibodies against the polypeptides for diagnostic, research or therapeutic purposes.

Groups I and VI are related insofar as the amount of the polypeptides of group I present in a cell may be used as a diagnostic for cancer or susceptibility to cancer. However, the polypeptides of group I can be used for a materially different purpose, such as for generating antibodies against the polypeptides for diagnostic, research or therapeutic purposes.

Groups II and III are related only insofar as an antibody directed against the polypeptides of group I can in principle also serve as an agonist or antagonist of the polypeptides. However, the groups are distinct because no such limitation is recited in the claim, and as such: (i) the compounds of group III can comprise molecules that are physically and chemically distinct from the antibody of group II and (ii) the antibody of group II can be used for a materially different purpose, such as for detecting the presence of the polypeptides of group I in a cell or tissue.

Groups II and IV are related only insofar as the antibody of group II could in principle be used as the compounds claimed in group IV. However, the groups are distinct for the same reasons given with respect to groups II and III above.

Groups II and V are related only insofar as the antibody of group II could in principle be identified using the method of group V. However, the groups are clearly distinct because (i) the method of group V is a generic method not limited to detection of antibodies and (ii) the antibody of group II can be used for a materially different purpose, such as for detecting the presence of the polypeptides of group I in a cell or tissue.

Groups II and VI are related insofar as the antibody of group II could in principle be used as the diagnostic agent to be employed in the process of group VI. However, the claims of group VI do not contain this limitation, and furthermore, (i) the process of group VI can employ other agents, such as a radiolabelled ligand for use in a binding assay, and (ii) the antibody of group II can be used for a materially different purpose, such as in an affinity column to purify the polypeptides of group I.

Groups III and IV are related insofar as the methods of treatment of group IV employ the compounds of group III. However, the groups are distinct because the compounds of group III can be used for materially different purposes, such as in the production of radiolabelled ligands for use in binding assays to detect levels of the polypeptides of group I.

Groups III and V are related only insofar as the compounds of group III may be detected using the method of group V. However, the groups are distinct because the compounds of group III can be used for materially different purposes, such as in the production of radiolabelled ligands for use in binding assays to detect levels of the polypeptides of group I. Furthermore, the compounds of group III can comprise a virtually infinite variety of molecules which could have a virtually infinite variety of materially different purposes, depending on the species of compound.

Groups III and VI are related insofar as at least some of the compounds of group III could in principle be used, for example in radiolabelled form, as the diagnostic agents in the process of group VI. However, the groups are distinct because the compounds of group III can comprise a virtually infinite variety of molecules which could have a virtually infinite variety of materially different purposes, depending on the species of compound.

Groups IV and VI are related only insofar as the methods of group IV employ compounds that interact with receptors whose levels are detected in the process of group VI. However, the groups are clearly distinct, as they are drawn to materially different methods with distinctly different objectives.

Groups V and VI are related insofar as at least some of the compounds identified using the method of group V could in principle be used, for example in radiolabelled form, as a diagnostic agent in the method of group VI. However, the groups are distinct because (i) the methods are materially distinct and have distinctly different (perhaps reciprocal) objectives and (ii) the compounds identified using the method of group V can be used for materially different purposes, for example, as a research tool to study structure-function relationships in TGF receptors.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.